# Effect of Processing Methods on Glycemic Index of Chocolate Crackers Made with Modified Kepok Banana (*Mussa paradisiaca* L.) Flour

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***Abstract*—Type 2 diabetes mellitus can be controlled or prevented by consuming foods which had a low glycemic index (GI). Modified kepok banana (Mussa paradisiaca L.) flour was made with an autoclaving-cooling method and spontaneous fermentation to produce low glycemic index chocolate crackers as a snack for type 2 diabetes patients. This study analyzed the organoleptic properties, the content of resistant starch and in vitro starch digestibility, the glycemic index [GI], and glycemic load [GL] of chocolate crackers made with modified kepok banana flour. The inclusion of modified kepok banana flour produced significant differences in color, texture and taste in the crackers, which also had a low GI and GL. In addition, the resistant starch content was unaffected by the production method, while the in vitro starch and digestibility were affected by the amount of kepok banana flour used.**

***Index Terms*—kepok banana, type 2 diabetes mellitus, resistant starch, in vitro starch digestibility, glycemic index.**

## I. Introduction

Type 2 diabetes mellitus is a metabolic syndrome that is characterized by hyperglycemia caused by abnormal insulin secretion [1]. In Indonesia, approximately 10 million people (or 6.2% of the population) have type 2 diabetes, which is the 6th highest worldwide after China, India, the United States, Brazil, Russia, and Mexico [2]. Type 2 diabetes mellitus can be controlled through dietary modifications, particularly the consumption of foods with a low GI that does not promote rapid increases in blood sugar levels [3]. Moreover, foods containing starches that are resistant to degradation by amylases require longer times to digest and in turn avoid spikes in blood glucose levels [4].

Kepok banana (Mussa paradisiaca L.) is a variety of banana that can be grown in a wide area of Indonesia. Kepok banana contain ~22% starch, of which ~28% is resistant to amylase-mediated degradation [5]. A previous study showed that the resistant starch content of bananas can be increased to 71.3% using an autoclaving-cooling method combined with spontaneous fermentation [6]. Meanwhile, the American Diabetes Association recommends that type 2 diabetes patients consume crackers because the dense calorie content of crackers can be used to control blood glucose levels [7]. Recently, the using of banana flour in biscuits were discovered can lower the GI [8].

Biscuits that were made of 15% banana flour had a GI value of 116, whereas biscuits that were made of 50% banana flour lowered the GI value to 98. In this study, we assessed the value of chocolate crackers made with kepok banana flour (which had a high content of resistant starch with a low GI) as an alternative snack for type 2 diabetes patients.

## II. Material and Methods

This study was conducted at Diponegoro University, Semarang and was approved by the health ethics committee at Dr. Moewardi Hospital/ Sebelas Maret University Faculty of Medicine Surakarta (Ethical Clearance No. 661/VII/HREC/2016).

### A. Modified Kepok Banana Flour

Unripe *kepok* bananas were obtained in traditional markets in Semarang and divided into three groups according to the method used to increase resistant starch content, including: 1) the autoclaving-cooling group, AC; 2) combination autoclaving-cooling with spontaneous fermentation group, ACF; and 3) no autoclaving-cooling with spontaneous fermentation group, NON. The AC

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group banana treatment involved heating with pressure of banana pulp in an autoclave to 121 ℃ for 15 minutes, followed by cooling for 24 hours at 4 ℃, the material was then dried. The ACF bananas were immersed in distilled water for 24 hours, after which the bananas were pressure heated with autoclave, then cooled, and dried. Bananas in the NON group were directly dried. The dried bananas were pulverized and passed through an 80 mesh sieve.

### B. Chocholate Crackers Modified With Kepok Banana Flour

Chocolate crackers were made by mixing the indicated amount of modified kepok banana flour, salt, wheat flour, stevia sugar, margarine, baking soda, yeast, cocoa powder, skim milk, and cold water to form smooth dough that was molded to form 1-2 mm thick sheets. The sheets were then cut into 2 x 2 cm squares that were baked at 100 ℃ for 20 minutes.

### C. Resistant Starch

A strach sample (0.5 g) was dissolved in 25 ml 80 mM sodium phosphate buffer (pH 6) and treated with 0.05 ml α-amylase (Termamyl by cargil) at 95 ℃ for 45 min. The mixture was cooled, centrifuged and the supernatant was discarded. The pellet was then hydrolyzed with 0.05 ml protease enzyme (40 mg protease enzyme/50 ml PBS, pH 6), and amyloglucosidase (5 ml HCl 0.325 N,pH 4.3) to remove proteins and hydrolyze starches. After an overnight incubation, the solution was filtered through Whatman filter paper 40. The residue was washed three times with ethanol and then dried [9]. The amount of resistant starch was calculated using:

$$\text{Resistant starch (\%)} = \frac{Residue\ (gr)}{sample\ (gr)} \text{x } 100\ \%. \qquad (1)$$

### D. In Vitro Starch Digesbility

Chocolate crackers sample (0.5 g) was first suspended in 50 ml water to 1%w/v and incubated at 90 ℃ for 30 mins to determine the in vitro starch digestibility. Then, 3 ml water was added to a 2 ml sample and 5 ml 100 mM sodium phosphate buffer (pH 7) was added and incubated with α-amylase at 37 ℃ for 30 mins. A 1 ml sample was then transferred into a new tube containing 2 ml dinitrosalicylic acid (DNS) and the mixture was heated to 100 ℃ for 10 minutes. After the incubation, the amount of red-orange color that formed was measured in a spectrophotometer at 520 nm. The maltose content was calculated using a standard curve generated using pure maltose obtained by reacting maltose solution with DNS reagents as described above. A sample containing only α-amylase was also measured, as was a solution lacking the enzyme and containing an equal volume of sodium phosphate buffer [10].

### E. GI Measurement and Subject Selection

Crackers with 50% substitution of kepok banana flour had the highest organoleptic performance and were used to measure the GI among the AC, ACF, and NON treatment methods. A minimum of 8 subjects were enrolled. All enrolled subjects met the following criteria, which were: 1) aged 18-30 years; 2) normal nutritional status with a BMI of 18.5 to 22.9 kg /m2; and 3) normal fasting blood glucose (FBG) of <100 mg/dl. Exclusion criteria were illness or prescription medication. All subjects provided written informed consent [11].

Subjects fasted for 10 hours and fasting blood glucose FBG values were determined the following morning. Subject blood samples (1-2 ml) were taken every 30 minutes for two hours (30, 60, 90 and 120 minutes). Blood glucose values were measured using Autocheck 3 in 1 glucometers (General Life Biotechnology Co., Ltd). The GI measurements were divided according to the three treatments: standard food treatment and two different testing food treatments. Each sample contained 50 grams of carbohydrates that were determined by testing samples with known levels of available carbohydrates using the by difference method [12]. Food standards that were used 50 grams pure glucose powder dissolved in 250 ml water [13]. Each treatment was done with the same subjects and spaced 3-5 days apart to avoid bias for any food tested.

### F. GI and Glycemic Load Measurement

GI was calculated using the incremental area under the blood glucose response curve (IAUC) method. Available carbohydrates were obtained by difference calculation. Blood glucose data from the subjects was plotted as a function of time. GI was calculated by comparing the AUC of test food with the standard food, and the results were averaged [11]. The GI was divided into three classifications [14]: low GI (<55), moderate (55-70), and high (> 70). The glycemic load (GL) was obtained by multiplying the available carbohydrate content of the GI value for a 25 g serving of crackers made with kepok banana flour and then divided by 100 [12]. GL values were grouped into three types [14]: BG low (≤ 10); BG medium (11-19); BG high (≥ 20).

### G. Data Analysis

Resistant starch content and starch digestibility in vitro was analyzed using a two-factor completely randomized design with nine treatments and two replications. The first factor was the three methods for producing kepok banana flour and the second factor was the amount of modified kepok banana flour that was substituted for wheat flour (25%, 50%, 75%). The data were analyzed using two way ANOVA followed by Tukey's test, whereas the preference level was analyzed using the Friedman test followed by Wilcoxon's test. GI and GL data were analyzed with descriptive statistics.

## III. Results And Discussion

### A. Resistant Starch

Resistant starch is defined as the starch that is not digested in the human gastrointestinal tract. Resistant starch is not hydrolyzed into D-Glucose in the small intestine within 120 minute of consumption, but instead is fermented by resident microflora of the colon [15].

Resistant starch is categorized by contents as:<1%, very low; 1-2.5%, low; 2.5% - 5% moderate; 5-15%, high; and >15%, very high [17]. Based on statistical analysis results in our study, the increased amounts of kepok banana flour that were used to produce chocolate crackers caused the resistant starch levels to increase, though there was no significant difference in resistant starch content among the kepok banana flour treatment methods (p = 0.620). Chocolate crackers made with 75% kepok banana flour using the ACF method had the highest resistant starch content (9%). Resistant starch content is influenced by the ratio of amylose and amylopectin starch and the crystallization process. The increasing levels of resistant starch in crackers (Table I.) can be influenced by interactions with other materials, such as protein and fat that are present during the manufacturing process as well as the production method used [5].

TABLE I. RESISTANT STARCH CONTENT AND IN VITRO DIGESTIBILITY OF CHOCOLATE CRACKERS MADE WITH MODIFIED KEPOK BANANA FLOUR SUBSTITUTION

| Method | Resistant starch | | | In vitro digestibility | | |
|---|---|---|---|---|---|---|
| | 25% | 50% | 75% | 25% | 50% | 75% |
| ACF | 1.9±0.21 | 5.18±0.85 | 8.97±1.36 | 55.77±1.93$^{g}$ | 31.93±0.11$^{d}$ | 21.97±3.74$^{a}$ |
| AC | 1.3±0.21 | 4.60±0.40 | 8.96±0.65 | 57.16±1.64$^{h}$ | 33.35±1.24$^{e}$ | 22.93±0.12$^{b}$ |
| NON | 1.28±0.17 | 4.28±2.15 | 8.47±1.81 | 57.68±0.60$^{h}$ | 40.31±1.25$^{f}$ | 23.49±2.35$^{c}$ |

Description: Values followed by different superscript letters (a-f ) showed significant differences.highest resistant starch content (9%).

## B. *In Vitro Digesbility*

The digestibility of starch reflects the ability of enzymes to degrade starches into simpler units. In vitro starch digestibility can be influenced by the amount of resistant starch. The digestion time increases as the levels of resistant starch increases, which avoids rapid increases in blood glucose levels [17].

Starch digestibility of kepok banana flour crackers is influenced by both intrinsic and extrinsic factors. The intrinsic factor are physical form of food and its digestion by pancreatic amylase. The extrinsic factors are the digestion time in the stomach (transit time), intestinal amylase activity and concentration, the amount of starch, and the presence of other food components [18].

The chocolate crackers made with 75% kepok banana flour substitution that were processed with ACF method had lowest in vitro digestibility of starch (22%) (Table I.), so the digestion of food will be slower and the activity of α-amylase will be decreased. Those effects will slow the absorption of glucose e [19]. The physiological effects of resistant starch on blood glucose levels can be explained by two mechanisms: the inhibition mechanism of α-amylase enzyme activity in the small intestine and the mechanism of increasing production of short chain fatty acids (SCFA) [20]. SCFA is the main product of dietary fiber and resistant starch degradation by anaerobic bacteria in the large intestine. SCFA may affect energy metabolism by delaying insulin secretion and decreasing glucose absorption to preserve glucose homeostasis [21].

## C. *Organoleptic Properties of Kepok Banana Flour Crackers*

Non-enzymatic browning reaction called the Maillard reaction may occur during the production of chocolate crackers containing modified banana flour [22]. It could influence the organoleptic properties of kepok banana flour crackers (Fig. 1). The overall color of the chocolate crackers containing the modified banana flour is dark brown [23]. Moreover, due to the low gluten content of kepok banana flour, chocolate crackers containing kepok banana flour had a hard texture and they can be easily broken [22]. These crackers also had a bland taste due the substitution of stevia for sucrose. In terms of the organoleptic properties taste, texture, and aroma, the AC group chocolate crackers with 50% substitution of banana flour kepok had the highest values, and also had the highest resistant starch content.

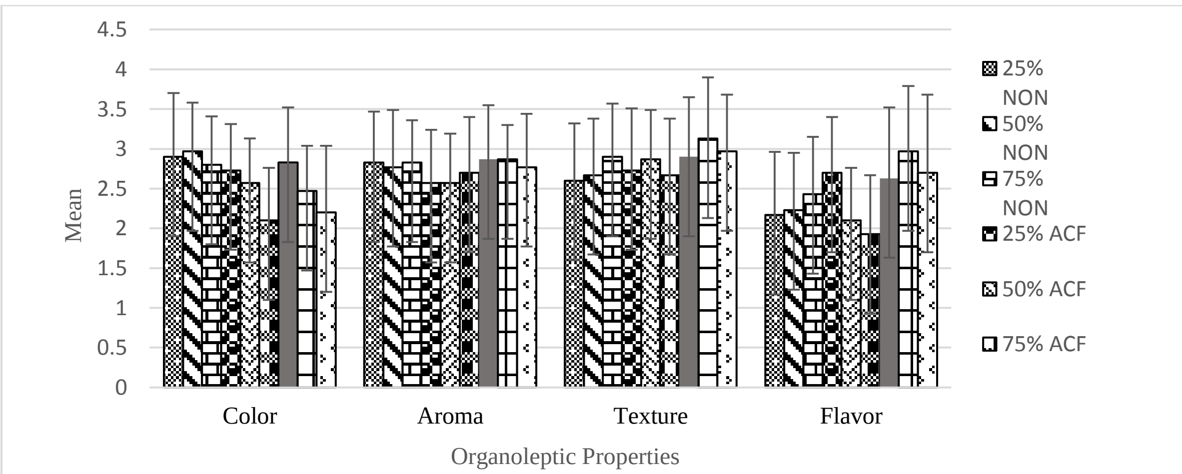


Figure 1. Organoleptic properties of chocolate crackers made with modified kapok banana flour substitution.

### D. Glycemic Index

Both AC and ACF group crackers had a low GI of < 55 [14]. Low GI food is absorbed slower in the small intestine and have a slower digestion rate. This occurs because of peak glucose circulation and insulin remains stable after a low GI meal and production of incretin hormones associated with stable glucose absorption [24]. Meanwhile, the NON group crackers with 50% kepok banana flour substitution had a higher GI compared to the ACF group (Table II).

Banana slices that were fermented with spontaneous fermentation and combined with one cycle of autoclaving-cooling increased the content of resistant starch in the resulting banana flour by more than 17% of the dry weight [6]. These results are consistent with an increase of 17.4% of resistant starch levels in the ACF group crackers made with 50% kepok banana substitution relative to those of the NON group. In vitro starch digestibility of ACF crackers was also decreased to 20.8% compared to NON group crackers made with 50% kepok banana flour substitution.

TABLE II. GLYCEMIC INDEX OF CHOCOLATE CRACKERS WITH *KEPOK* BANANA FLOUR SUBSTITUTION

| Chocolate crackers with *kepok* banana flour (%substitution) | Carbohydrate by difference | Cracker Sample (g) | Area under the curve ($cm^2$) | Glycemic index (%) | Category* |
|---|---|---|---|---|---|
| 50% NON | 66.26 | 75.46 | 2735.6 | 50.06 ±14.78 | Low |
| 50% ACF | 75.46 | 67.76 | 2261.9 | 38.42 ±15.05 | Low |

* Category : low GI (<55), medium GI (55-70), high GI (>70)

### E. Glycemic Load

The GL describes blood glucose enhancement based on carbohydrate content contained in one serving of food [14]. Both types of crackers (AC and ACF) in this study had a lower GL relative to NON crackers (Table III.) Thus, kepok banana flour produced by autoclaving-cooling followed by fermentation may be a useful ingredient to produce low-GI and low-GL snacks for type 2 diabetes patients.

TABLE III. GLYCEMIC LOAD OF CHOCOLATE CRACKERS WITH *KEPOK* BANANA FLOUR SUBSTITUTION

| Chocolate Crackers with *Kepok* Banana Substitution | Serving size(g) | Available Carbohydrate (%) | Available Carbohydrate/ serving (%) | Glycemic Load | Category |
|---|---|---|---|---|---|
| 50% NON | 25 | 66.26 | 16.57 | 8.29 ± 2.45 | Low |
| 50% ACF | 25 | 73.78 | 18.45 | 7.09 ±2.78 | Low |

* Available Carbohydrate/serving (%) = $\frac{serving\ size\ (gram)\ X\ available\ carbohydrate}{100\ gram}$

** Glycemic Load = $\frac{GI\ X\ totsl\ available\ carbohydrate\ perserving}{100}$

*** Category : low GL (<10), medium GL (11-19), high GL (>20)

## IV. CONCLUSIONS

Chocolate crackers made with 75% kepok banana flour using the ACF method had the highest resistant starch content (9%) and lowest in vitro digestibility of starch (22%). In terms of the organoleptic properties taste, texture, and aroma, the AC group chocolate crackers with 50% sub-stitution of banana flour kepok had the highest values. Both AC and ACF group crackers had a low GI of < 55 and a lower GL relative to NON crackers. Kepok banana flour produced by autoclaving-cooling followed by fermentation may be a useful ingredient to produce low-GI and low-GL snacks for type 2 diabetes patients.

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